

### **LISTING OF THE CLAIMS**

The following is a complete listing of the claims.

#### **LISTING OF THE CLAIMS**

1. (PREVIOUSLY PRESENTED) A method for detecting contaminants in a medium suspected of containing such contaminants, the method comprising the consecutive steps of:
  - a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device;
  - b) contacting the influent side of the filter in the filter device with a liquid vehicle containing at least one substrate, wherein said at least one substrate through interaction with an enzyme characteristic of the contaminants produces a detectable moiety;
  - c) allowing the substrate to interact with the contaminants on the influent side of the filter in the filter device for a period of time, which is sufficient to allow the detectable moiety to be detected in the liquid vehicle;
  - d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter; and
  - e) performing a quantitative or qualitative detection of the detectable moiety in the liquid vehicle evacuated in step d and correlating the detection of the moiety to the amount or presence of contaminants in the sample.
2. (ORIGINAL) The method according to claim 1, wherein, prior to step a, the medium is passed through a prefilter that does not retain the contaminants, but retains larger particles.

3. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the contaminants are selected from the group consisting of bacteria; fungi; algae; protozoans; spores from bacteria; fungal spores; and pollen, and fragments thereof.
4. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the medium is a liquid medium.
5. (ORIGINAL) The method according to claim 4, wherein the liquid medium is selected from the group consisting of environmental water, drinking water, hot water, industrial water, process water, cleaning in place water, a liquid extract of a solid material, a suspended or solubilised surface sample, and liquid industrial products such as cosmetics, pharmaceuticals, and foodstuffs.
6. (PREVIOUSLY PRESENTED) The method according to claim 4, wherein the viscosity of the liquid medium is reduced prior to step a.
7. (PREVIOUSLY PRESENTED) The method according to claim 6, wherein the viscosity is reduced by means of dilution or by means of treatment with a chemical agent.
8. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the medium is a gaseous medium.
9. (PREVIOUSLY PRESENTED) The method according to claim 8, wherein the gaseous medium is air.

10. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the filter has a pore size small enough so as to retain substantially all contaminants in the medium.
11. (ORIGINAL) The method according to claim 10, wherein the filter has a pore size large enough to let the detectable moiety pass through the filter.
12. (ORIGINAL) The method according to claim 11, wherein the pore size is at most 20  $\mu\text{m}$ .
13. (PREVIOUSLY PRESENTED) The method according to claim 11, wherein the pore size is at least 0.1  $\mu\text{m}$ .
14. (CANCELLED)
15. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the enzyme is selected from the group consisting of carbohydrases, proteases, lipases, esterases, amidases, sulfatases, nucleases and phosphatases.
16. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the enzyme is expressed constitutively by microorganisms.
17. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the at least one substrate is a fluorogenic or chromogenic substrate producing blue, green and red products as the detectable moiety.

18. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the at least one substrate is selected from the group consisting of 5-bromo-4-chloro-3-indolyl phosphate disodium salt; 9h-(1,3-dichloro-9,9-dimethylacridine-2-one-7-yl) phosphate ammonium salt; fluorescein diphosphate tetraammonium salt; a methylumbelliferyl derivative; salts of 4-nitrophenyl phosphate; and resorufin phosphate.

19. (CANCELLED)

20. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the at least one substrate includes at least two substrates that produce detectable moieties providing signals that can be combined into one single measured signal value.

21. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the at least one substrate includes at least two substrates that produce detectable moieties providing distinguishable signals.

22. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the contaminants are viable microorganisms.

23. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the amount of the at least one substrate in the liquid vehicle does not limit the rate of production of the detectable moiety.

24. (ORIGINAL) The method according to claim 23, wherein the rate of production of the detectable moiety is a function of the quantity of contaminants in the known volume of the medium.

25. (ORIGINAL) The method according to claim 24, wherein the function is linear.

26. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein several different known volumes of the medium are each passed through a filter in step a, so as to ensure that at least one of the volumes contains a suitable number of the contaminants.

27. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the filter is part of a closed, sterile filter device.

28. (ORIGINAL) The method according to claim 27, wherein the closed, sterile filter device is disposable.

29. (PREVIOUSLY PRESENTED) The method according to claim 27, wherein the closed, sterile filter device integrates the filter and a filter housing into one irreversibly closed structural unit.

30. (PREVIOUSLY PRESENTED) The method according to claim 27, wherein the longest cross-sectional axis of the closed, sterile filter device does not exceed a length of 10 cm.

31.-33. (CANCELLED)

34. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein evacuation is obtained by applying an elevated pressure on the influent side of the filter or by applying a lowered pressure on the effluent side of the filter.

35.-36. (CANCELLED)

37. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein detection in step d is performed by measuring fluorescence characteristic of the detectable moiety.

38. (PREVIOUSLY PRESENTED) The method according to claim 37, wherein the fluorescence in step d is measured directly in the liquid vehicle without an interruption of the contact between the liquid vehicle and the contaminants.

39. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the correlation in step d comprises the use of a pre-determined standard curve that expresses the relationship between the amount of contaminants and the amount of the detectable moiety under standard conditions.

40. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein detection is performed in a microtiter system.

41. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein the contaminants are subjected to a signal-enhancing influence, either prior to step a or in step b, and

the signal-enhancing influence is one selected from the group consisting of an enzyme-enhancing substance, a selective temperature, a temperature range, a selective pH, a selective salt concentration, a non-selective growth-enhancer and a selective growth-enhancing substance.

42. (ORIGINAL) The method according to claim 41, where the signal-enhancing influence increases the overall sensitivity in a subsequent detection or favours subsequent detection of specific types of contaminants, or reduces detection of specific types of contaminants.

43. (CANCELLED)

44. (PREVIOUSLY PRESENTED) The method according to claim 1, wherein step a is preceded by an incubation of the medium.

45. (PREVIOUSLY PRESENTED) The method according to claim 44, wherein the incubation entails at least one selected from the group consisting of:

- treatment with an enzyme inducing substance thereby enhancing the detection of the detectable moiety,
- subjecting the medium to a selective substance for yeast, fungi or bacteria,
- subjecting the medium to a non-selective growth-enhancer for microorganisms, and
- subjecting the medium to a substance capable of extracting cellular enzymes.

46.-47. (CANCELLED)

48. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the detectable moiety is detectable in an amount of at the most 50 picomoles.

49. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the detectable moiety is detectable in an amount of at the most 20 picomoles.

50. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the detectable moiety is detectable in an amount of at the most 10 picomoles.

51. (PREVIOUSLY PRESENTED) The method according to claim 57, wherein the detectable moiety is detectable in an amount of at the most 1 picomoles.

52. (PREVIOUSLY PRESENTED) The method according to claim 3, wherein the fungi is one selected from the group consisting of filamentous fungi and yeast.

53. (PREVIOUSLY PRESENTED) The method according to claim 7, wherein the chemical agent is one selected from the group consisting of a solubility enhancing agent and a detergent.

54. (PREVIOUSLY PRESENTED) The method according to claim 9, where the air is one selected from the group consisting of air from sterile facility, air from a laminar air-flow device and environmental air.



55. (PREVIOUSLY PRESENTED) The method according to claim 15, wherein the enzyme is a phosphatase, and the phosphatase is alkaline phosphatase.

56. (PREVIOUSLY PRESENTED) The method according to claim 18, wherein the methylumbelliferyl derivative is one selected from the group consisting of 6,8-difluoro-4-methylumbelliferyl phosphate, 4-methylumbelliferyl phosphate dicyclohexylammonium salt trihydrate, 4-methylumbelliferyl phosphate free acid; 4-methylumbelliferyl phosphate dilithium salt, 4-methylumbelliferyl- $\beta$ -N-acetylglucosaminide and trifluoromethylumbelliferyl phosphate.

57. (PREVIOUSLY PRESENTED) The method of claim 1, wherein the at least one substrate produces the detectable moiety by being cleaved by an enzyme that is characteristic for the contaminants.

58. (PREVIOUSLY PRESENTED) The method of claim 57, wherein the detectable moiety is detectable in an amount of at the most 100 picomoles.

59. (PREVIOUSLY PRESENTED) A method for detecting contaminants in a medium suspected of containing such contaminants, the method comprising the consecutive steps of:

a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device;

b) contacting the influent side of the filter in the filter device with a liquid vehicle containing at least one substrate, wherein said at least one substrate through

interaction with an enzyme characteristic of the contaminants produces a detectable moiety;

c) allowing the substrate to interact with the contaminants on the influent side of the filter in the filter device for a period of time between 5-minutes to 24-hours, which is sufficient to allow the detectable moiety to be detected in the liquid vehicle;

d) evacuating the liquid vehicle from the influent side of the filter by forcing the liquid vehicle through to the effluent side of the filter; and

e) performing a quantitative or qualitative detection of the detectable moiety in the liquid vehicle evacuated in step d and correlating the detection of the moiety to the amount or presence of contaminants in the sample.

60. (PREVIOUSLY PRESENTED) The method of claim 59, wherein the period of time is between 20-minutes to 12-hours.

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END OF CLAIM LISTING